

**Mobile phase:** Cyclohexane:ethyl acetate 40:60

**Injection volume:** 200

**Detector:** F ex 247 em 547, after post-column reaction with 30 mM Tb(NO<sub>3</sub>)<sub>3</sub> in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

---

#### CHROMATOGRAM

**Retention time:** 12

**Limit of detection:** 80 pg/mL

---

#### OTHER SUBSTANCES

**Extracted:** testosterone acetate, bolasterone, testosterone

**Simultaneous:** 17-methyltestosterone

---

#### KEY WORDS

SPE; normal phase; post-column reaction

---

#### REFERENCE

Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by sensitized terbium fluorescence, *Anal.Chem.*, **1993**, 65, 2346–2351.

---

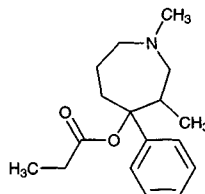
## Proheptazine

**Molecular formula:** C<sub>17</sub>H<sub>25</sub>NO<sub>2</sub>

**Molecular weight:** 275.39

**CAS Registry No.:** 77-14-5

**Merck Index:** 7959



---

#### SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

#### HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

---

#### CHROMATOGRAM

**Retention time:** 3.9

---

#### OTHER SUBSTANCES

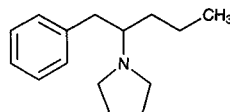
**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

# Prolintane



**Molecular formula:** C<sub>15</sub>H<sub>23</sub>N

**Molecular weight:** 217.35

**CAS Registry No.:** 493-92-5, 1211-28-5 (HCl)

**Merck Index:** 7964

**Lednicer No.:** 1 70

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 2.7

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextro-

propoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mepitazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylethylgonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenylamine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelethamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191-225.

# Promazine

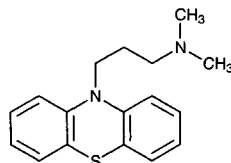
**Molecular formula:** C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>S

**Molecular weight:** 284.43

**CAS Registry No.:** 58-40-2, 53-60-1 (HCl)

**Merck Index:** 7966

**Lednicer No.:** 1 377



## SAMPLE

**Matrix:** blood

**Sample preparation:** 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 µL mobile phase, inject a 50 µL aliquot.

## HPLC VARIABLES

**Column:** 10 µm Micropak CN (Varian)

**Mobile phase:** MeCN:5 mM ammonium acetate 90:10

**Flow rate:** 2.5

**Injection volume:** 50

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 27.5

**Limit of detection:** 10 ng/mL

---

**OTHER SUBSTANCES**

**Simultaneous:** acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, trifluoperazine, chlorpromazine, thiothixene, thioridazine, triflupromazine, trihexyphenidyl, trimeprazine, metabolites

---

**KEY WORDS**

plasma

---

**REFERENCE**

Curry, S.H.; Brown, E.A.; Hu, O.Y.-P.; Perrin, J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, 231, 361-376.

---

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200  $\mu$ L 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200  $\mu$ L mobile phase, vortex 10 s, inject 75-100  $\mu$ L aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25  $\mu$ L concentrated HCl.)

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelco silica

**Mobile phase:** EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)

**Flow rate:** 2

**Injection volume:** 75-100

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 5.2

**Internal standard:** promazine

---

**OTHER SUBSTANCES**

**Extracted:** amitriptyline, desipramine, desmethyldoxepin, doxepin, imipramine, nortriptyline, protriptyline

**Simultaneous:** thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine, iprindole, pyrilamine, promethazine, prolixin, amoxapine, N-acetylprocainamide, procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxy-desipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine

**Noninterfering:** thiopropazine

---

**KEY WORDS**

serum; normal phase; promazine is IS

---

**REFERENCE**

Beierle, F.A.; Hubbard, R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, 5, 279-292.

---

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L

methanol. Elute with three aliquots of 300  $\mu\text{L}$  0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300  $\mu\text{L}$  0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100  $\mu\text{L}$  aliquot of the aqueous phase.

---

#### HPLC VARIABLES

**Guard column:** LC-8-DB (Supelco)

**Column:** 150  $\times$  4.6 LC-8-DB (Supelco)

**Mobile phase:** MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

**Flow rate:** 2

**Injection volume:** 100

**Detector:** UV 228

---

#### CHROMATOGRAM

**Retention time:** 3.9

---

#### OTHER SUBSTANCES

**Extracted:** acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil

**Noninterfering:** acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, ben-droflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocinide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

**Interfering:** desipramine, methaqualone, norverapamil, ibuprofen, propafenone, protriptyline

---

#### KEY WORDS

plasma; SPE

---

#### REFERENCE

Nichols, J.H.; Charlson, J.R.; Lawson, G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin. Chem.*, **1994**, *40*, 1312–1316.

---

#### SAMPLE

**Matrix:** formulations

**Sample preparation:** Tablets. Grind 5 tablets to a fine powder, dissolve in 100 mL MeOH:0.5% acetic acid 1:1, filter (paper), inject an aliquot. Suppositories. Cut up 3 suppositories, add to 100 mL MeOH:0.5% acetic acid 1:1, heat at 40° until all the fat melted, shake, filter (paper), inject a 25  $\mu\text{L}$  aliquot. Liquid formulations. Dilute 10 mL formulation to 100 mL with MeOH:0.5% acetic acid 1:1, inject a 25  $\mu\text{L}$  aliquot.

---

#### HPLC VARIABLES

**Column:** 300  $\times$  4  $\mu\text{m}$  Bondapak phenyl

**Mobile phase:** Gradient. A was 10 mM heptanesulfonic acid in 1 mM acetic acid. B was 10 mM heptanesulfonic acid and 1 mM acetic acid in MeOH. A:B from 60:40 to 25:75 over 30 min

**Column temperature:** 35

**Flow rate:** 1.75

**Injection volume:** 25

**Detector:** UV 225

---

**CHROMATOGRAM****Retention time:** 20

---

**OTHER SUBSTANCES****Simultaneous:** diphenhydramine, dipyrone (metamizol), adiphenine, ethyldiphenacetate, drofenine, impurities

---

**KEY WORDS**

tablets; suppositories; liquid formulations

---

**REFERENCE**Facchini,G.; Zaccheo,F.; Nannetti,M. Simultaneous determination of hydrochloride salts of adiphenine, diphenhydramine, ethyldiphenacetate, drofenine and promazine by ion-pair HPLC, *Boll.Chim.Farm.*, **1983**, 122, 405–411.

---

**SAMPLE****Matrix:** formulations**Sample preparation:** Extract ground tablets containing 1 mg with 10 mL MeOH, shake for 30 min, centrifuge at 2000 rpm for 5 min. Remove a 5 mL aliquot of the supernatant and add it to 10 mL 1.25 mg/mL norephedrine hydrochloride in MeOH, make up to 25 mL with MeOH, inject a 10  $\mu$ L aliquot.

---

**HPLC VARIABLES****Column:** 150  $\times$  4.6 5  $\mu$ m Zorbax CN**Mobile phase:** MeOH:MeCN:25 mM pH 4.5 acetate buffer 30:40:30**Flow rate:** 2.5**Injection volume:** 10**Detector:** UV 254

---

**CHROMATOGRAM****Retention time:** 4.45**Internal standard:** norephedrine (2.38)

---

**OTHER SUBSTANCES****Interfering:** desipramine, fluphenazine

---

**KEY WORDS**

tablets

---

**REFERENCE**Beaulieu,N.; Gagné,C.; Lovering,E.G. Liquid chromatographic determination of identity, content, and content uniformity of desipramine, fluphenazine, and promazine, *J.Assoc.Off.Anal.Chem.*, **1986**, 69, 178–179.

---

**SAMPLE****Matrix:** formulations**Sample preparation:** Dissolve crushed tablets, suspensions, or injections in water to give a promazine concentration of 200  $\mu$ g/mL, inject a 20  $\mu$ L aliquot.

---

**HPLC VARIABLES****Column:** 250  $\times$  4.5 5  $\mu$ m Spherisorb silica**Mobile phase:** MeOH:buffer 75:25 (Buffer was 5% ammonium acetate adjusted to pH 9.5 with ammonia solution.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 240

---

**CHROMATOGRAM****Retention time:** 3**Limit of detection:** 1 ng

---

**OTHER SUBSTANCES**

**Simultaneous:** degradation products

---

**KEY WORDS**

tablets; injections; suspensions

---

**REFERENCE**

Tebbett,I.R. Analysis of promazine in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, 356, 227–229.

---

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Guard column:** 4 × 4 5 µm LiChroCART LiChrospher 60 RP Select B

**Column:** 125 × 4 5 µm LiChroCART LiChrospher 60 RP Select B

**Mobile phase:** MeCN:buffer 10:90 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 3.39

---

**OTHER SUBSTANCES**

**Simultaneous:** amiodarone

---

**REFERENCE**

Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, 728, 307–310.

---

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250 × 4 ODS (Hitachi)

**Mobile phase:** MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl

**Column temperature:** 55

**Flow rate:** 0.6

**Injection volume:** 20

**Detector:** UV 251

---

**OTHER SUBSTANCES**

**Also analyzed:** amitriptyline, chlorpromazine, clomipramine, promethazine, thymol

---

**REFERENCE**

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, 87, 960–966.

---

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 6.2**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promethazine, pronethalol, propidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

**SAMPLE****Matrix:** solutions**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.**HPLC VARIABLES****Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV**CHROMATOGRAM****Retention time:** k' 1.92



---

**REFERENCE**

Roos, R.W.; Lau-Cam, C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, 370, 403-418.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Guard column:** 30 × 2.1 Spheri-5 RP-8

**Column:** 220 × 2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

---

**CHROMATOGRAM**

**Retention time:** 13.5

---

**OTHER SUBSTANCES**

**Simultaneous:** mesoridazine, thiothixene, chlorpromazine, trifluoperazine, thioridazine

**Also analyzed:** amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

---

**REFERENCE**

*Rainin Catalog*, C1-94, 1994, p. 7.24.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Guard column:** 30 × 3.2 7 µm SI 100 ODS (not commercially available)

**Column:** 150 × 3.2 7 µm SI 100 ODS (not commercially available)

**Mobile phase:** MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH<sub>2</sub>PO<sub>4</sub> and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

**Flow rate:** 0.5-1

**Detector:** UV 247

---

**CHROMATOGRAM**

**Retention time:** 3.3

**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

---

**OTHER SUBSTANCES**

**Also analyzed:** aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

---

**REFERENCE**

Below, E.; Burrmann, M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J. Liq. Chromatogr.*, **1994**, 17, 4131-4144.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Lichrosphere cyanopropyl

**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 94:6:0.03

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 5

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 4.4

## OTHER SUBSTANCES

**Simultaneous:** triflupromazine, carphenazine, methotrimeprazine, perphenazine, chlorprothixene, deserpidine, thiothixene, reserpine

**Also analyzed:** acetophenazine, ethopropazine, promethazine, propiomazine

## KEY WORDS

SFC; pressure 200 bar

## REFERENCE

Berger, T.A.; Wilson, W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 14.24 (A), 6.34 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatidine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimizide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital,

sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

---

**KEY WORDS**

details of plasma extraction

---

**REFERENCE**

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 20  $\mu\text{L}$  aliquot of a 100–500  $\mu\text{g/mL}$  solution in mobile phase.

---

**HPLC VARIABLES**

**Column:** 100  $\times$  4.6 5  $\mu\text{m}$  Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

**Mobile phase:** MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60

**Flow rate:** 0.5–2

**Injection volume:** 20

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:**  $k'$  7.94

---

**OTHER SUBSTANCES**

**Also analyzed:** amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyrindamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

**Noninterfering:** acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

---

**KEY WORDS**

comparison with capillary electrophoresis

---

**REFERENCE**

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, 70, 2092–2099.

---

# Promethazine

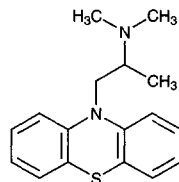
**Molecular formula:**  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{S}$

**Molecular weight:** 284.43

**CAS Registry No.:** 60-87-7, 58-33-3 (HCl), 17693-51-5 (teoclate)

**Merck Index:** 7970

**Lednicer No.:** 1 373



---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Attach PLUS™-MP3 extraction discs (15 mg/3 cc size, Ansys. Inc., CA) to a vacuum manifold and condition with 200  $\mu$ L MeOH followed by 400  $\mu$ L 100 mM pH 6.0  $\text{KH}_2\text{PO}_4$ . Dilute 1 mL serum containing 6000 ng verapamil with 1.5 mL MeOH:100 mM pH 6.0  $\text{KH}_2\text{PO}_4$  40:100, mix. Add the sample to the extraction disc by applying a vacuum of about 2 kPa. After aspirating the sample through the disc wash with two 300  $\mu$ L portions of MeOH: water 1:2, dry the disc under full vacuum for at least 5 min, elute with four 300  $\mu$ L portions of freshly prepared MeCN:triethylamine 100:2, evaporate the eluate to dryness under nitrogen, redissolve the residue in 800  $\mu$ L mobile phase, inject 100  $\mu$ L aliquot.

---

#### HPLC VARIABLES

**Guard column:** 15  $\times$  1 RP C8 (Optimize Technologies, OR)  
**Column:** 150  $\times$  4.6 5  $\mu$ m Chiralcel OJ-R (Chiral Technologies, PA)  
**Mobile phase:** MeCN:500 mM sodium perchlorate 37:63  
**Flow rate:** 0.5  
**Injection volume:** 100  
**Detector:** UV 249

---

#### CHROMATOGRAM

**Retention time:** 12.2 (R(+)), 14.1 (S(-))  
**Internal standard:** verapamil (9.5)  
**Limit of detection:** 2 ng/mL  
**Limit of quantitation:** 10 ng/mL

---

#### KEY WORDS

chiral; serum; SPE

---

#### REFERENCE

Liu, J.; Stewart, J.T. Quantitation of promethazine enantiomers in human serum using a chiralcel OJ-R column and mixed-mode disc solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1997**, 16, 303–309.

---

#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

---

#### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18  
**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)  
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.  
**Column temperature:** 30  
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)  
**Injection volume:** 10–30  
**Detector:** UV 251.1

---

#### CHROMATOGRAM

**Retention time:** 14.482

---

#### KEY WORDS

whole blood

---

#### REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250 × 4 ODS (Hitachi)

**Mobile phase:** MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl

**Column temperature:** 55

**Flow rate:** 0.6

**Injection volume:** 20

**Detector:** UV 251

---

**OTHER SUBSTANCES**

**Also analyzed:** amitriptyline, chlorpromazine, clomipramine, promazine, thymol

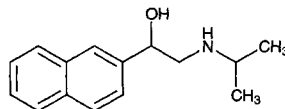
---

**REFERENCE**

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, 87, 960-966.

---

# Pronethalol



**Molecular formula:** C<sub>15</sub>H<sub>19</sub>NO

**Molecular weight:** 229.32

**CAS Registry No.:** 54-80-8. 51-02-5 (HCl)

**Merck Index:** 7974

**Lednicer No.:** 1 66

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

---

**CHROMATOGRAM**

**Retention time:** 2.0

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipamone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, hal-

operidol, hydroxyzine, hyoscyne, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylethylgonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelethamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191-225.

# Propafenone

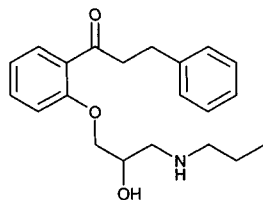
**Molecular formula:**  $C_{21}H_{27}NO_3$

**Molecular weight:** 341.45

**CAS Registry No.:** 54063-53-5, 34183-22-7 (HCl)

**Merck Index:** 7978

**Lednicer No.:** 5 17



## SAMPLE

**Matrix:** blood

**Sample preparation:** Mix 400  $\mu$ L serum with 20  $\mu$ L 10  $\mu$ g/mL IS in MeOH and 50  $\mu$ L 10% sodium carbonate. Add 4 mL diisopropyl ether, shake vigorously for 4 min, centrifuge, freeze at  $-20^\circ$ . Mix the organic layer with 100  $\mu$ L 10 mM HCl, vortex carefully for 45 s using a microshaker, centrifuge, evaporate the aqueous phase to dryness under a stream of argon in a  $56^\circ$  water bath. Reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

## HPLC VARIABLES

**Guard column:** 20  $\times$  4.6 5  $\mu$ m Supelguard LC-CN

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil LC-CN

**Mobile phase:** MeCN:water:500 mM  $KH_2PO_4$ , 36:62:2

**Flow rate:** 1.8

**Injection volume:** 50

**Detector:** UV 210

## CHROMATOGRAM

**Retention time:** 9.3

**Internal standard:** LU41616 (2'-[2-hydroxy-3-(3''-hydroxy-3''-methylbutylamino)propoxy]-3-phenylpropiophenone hydrochloride) (7.7)

**Limit of detection:** 5 ng/mL

**Limit of quantitation:** 20 ng/mL

---

**OTHER SUBSTANCES**

**Extracted:** metabolites, diltiazem, mexiletine

**Simultaneous:** acebutolol, amiodarone, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, flecainide, gallopamil, hexobarbital, lidocaine, mephenytoin, metoprolol, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propranolol, quinidine, sotalol, theophylline, verapamil

---

**KEY WORDS**

serum

---

**REFERENCE**

Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

---

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a 6 mL 500 mg ENVI-18 (Supelco) SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 25  $\mu$ L 100  $\mu$ g/mL IS, add to the SPE cartridge, wash with 5 mL water and 1 mL MeOH, dry under vacuum for 10 min, elute with 2 mL 700 mM ammonium hydroxide in MeOH, evaporate the eluate to dryness under a stream of air. Reconstitute the residue in 100  $\mu$ L mobile phase and 100  $\mu$ L hexane, vortex for 1 min, centrifuge at 1800 g for 5 min, inject a 20  $\mu$ L aliquot of the lower phase.

---

**HPLC VARIABLES**

**Guard column:** 4  $\times$  4 RP-8 endcapped (Merck)

**Column:** 250  $\times$  4.6 10  $\mu$ m Chiracel OD-R

**Mobile phase:** MeCN:250 mM sodium perchlorate adjusted to pH 4.0 with perchloric acid 40:60

**Flow rate:** 0.7

**Injection volume:** 20

**Detector:** UV 300

---

**CHROMATOGRAM**

**Retention time:** 16.5 (S), 18 (R)

**Internal standard:** propranolol (9, 12 (enantiomers))

**Limit of quantitation:** 25 ng/mL

---

**OTHER SUBSTANCES**

**Simultaneous:** atenolol, bromazepam, carbamazepine, clobazam, clonazepam, dexamethasone, diazepam, diclofenac, flunitrazepam, flurazepam, lidocaine, lorazepam, mebendazole, metoprolol, oxyphenbutazone, phenytoin, praziquantel, procainamide, propoxyphene, salicylic acid, triazolam, trimethoprim, verapamil, warfarin

**Noninterfering:** acetaminophen, albendazole, albuterol, alprazolam, cimetidine, disopyramide, fenfluramine, mexiletine, phenobarbital, pindolol, primidone

**Interfering:** amitriptyline, diltiazem, haloperidol, imipramine

---

**KEY WORDS**

plasma; chiral; SPE; pharmacokinetics

---

**REFERENCE**

de Gaitani,C.M.; Lanchote,V.L.; Bonato,P.S. Enantioselective analysis of propafenone in plasma using a polysaccharide-based chiral stationary phase under reversed-phase conditions, *J.Chromatogr.B*, **1998**, *708*, 177–183.

---

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 500  $\mu$ L Serum + 250  $\mu$ L di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50  $\mu$ L aliquot of top organic layer.

---

**HPLC VARIABLES**

**Guard column:** 30  $\times$  4.6 5  $\mu$ m Brownlee cyano spheri-5

**Column:** 250 × 4.6 5 µm Altex ultrasphere cyano

**Mobile phase:** MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5

**Column temperature:** 20

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 248

---

#### CHROMATOGRAM

**Retention time:** 6.5

**Internal standard:** minaprine (5.5)

**Limit of detection:** 50 ng/mL

---

#### OTHER SUBSTANCES

**Simultaneous:** amiodarone, desethylamiodarone, diltiazem, verapamil, nortriptyline, amitriptyline

**Also analyzed:** haloperidol, desipramine, imipramine, clomipramine

---

#### KEY WORDS

serum

---

#### REFERENCE

Mazzi, G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

---

#### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 100 µL 1 µg/mL (-)-ephedrine in water + 200 µL saturated sodium carbonate + 4 mL hexane:isopropanol:heptafluoro-1-butanol 95:5:1.25, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL reagent, vortex for 5 s, let stand at room temperature for 3 min, add 100 µL bupranolol solution, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 400 µL hexane, add 200 µL 100 mM HCl, vortex for 15 s, centrifuge at 1800 g for 5 min, inject a 100 µL aliquot of the upper organic layer. (Prepare reagent by diluting commercially available R-(-)-1-(1-naphthyl)ethyl isocyanate 10-fold with hexane, pass through a 50 mm column of silica, dilute the eluate with hexane to a concentration of 0.1%, store in amber containers at -30°. Prepare working reagent immediately before use by diluting to 0.005% with hexane:isopropanol 95:5. Prepare bupranolol solution by dissolving bupranolol hydrochloride in 1 mL water, add 500 µL saturated sodium carbonate solution, extract with 25 mL hexane, use the hexane solution.)

---

#### HPLC VARIABLES

**Guard column:** 50 × 4.6 pellicular silica (Whatman)

**Column:** 100 × 4.6 5 µm Partisil 5 silica

**Mobile phase:** Hexane:isopropanol:isobutanol 96:2:2

**Flow rate:** 1.5

**Injection volume:** 100

**Detector:** UV 220

---

#### CHROMATOGRAM

**Retention time:** 8.3 (-), 10.1 (+)

**Internal standard:** (-)-ephedrine (16.4)

**Limit of quantitation:** 6.25 ng/mL

---

#### OTHER SUBSTANCES

**Simultaneous:** alprenolol, methoxamine, mexiletine, pindolol, propranolol, tocainide

---

#### KEY WORDS

plasma; normal phase; derivatization; pharmacokinetics; chiral

---

#### REFERENCE

Mehvar, R. Liquid chromatographic analysis of propafenone enantiomers in human plasma, *J. Chromatogr.*, **1990**, *527*, 79–89.



---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 200  $\mu$ L 1 M NaOH + 5 mL dichloromethane, mix for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in hexane:isopropanol 90:10, inject an aliquot.

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 10  $\mu$ m Chiralpak AD (J.T. baker)

**Mobile phase:** n-Hexane:isopropanol:diethylamine 75:25:0.2

**Flow rate:** 1

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 6.5 (R-(-)), 9 (S-(+))

---

**KEY WORDS**

plasma; chiral

---

**REFERENCE**

Hollenhorst,T.; Blaschke,G. Direct separation of the enantiomers of propafenone, diprafenone and their major metabolites by high-performance liquid chromatography on modified cellulose and amylose chiral stationary phases, *J.Chromatogr.*, **1991**, 585, 329–332.

---

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300  $\mu$ L 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300  $\mu$ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100  $\mu$ L aliquot of the aqueous phase.

---

**HPLC VARIABLES**

**Guard column:** LC-8-DB (Supelco)

**Column:** 150  $\times$  4.6 LC-8-DB (Supelco)

**Mobile phase:** MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

**Flow rate:** 2

**Injection volume:** 100

**Detector:** UV 228

---

**CHROMATOGRAM**

**Retention time:** 4.2

---

**OTHER SUBSTANCES**

**Extracted:** acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil

**Noninterfering:** acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, ben-droflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide,

primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocinide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

**Interfering:** desipramine, methaqualone, norverapamil, ibuprofen, promazine, protriptyline

---

**KEY WORDS**

plasma; SPE

---

**REFERENCE**

Nichols, J.H.; Charlson, J.R.; Lawson, G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin. Chem.*, **1994**, *40*, 1312–1316.

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Centrifuge plasma at 1439 g for 10 min. 1 mL Plasma + 50  $\mu$ L n-hexane:isopropanol containing 8  $\mu$ g/mL R,S-propranolol hydrochloride and 20  $\mu$ g/mL R,S-metoprolol tartrate + 200  $\mu$ L pH 11 ammonium chloride/ammonium hydroxide buffer + 3 mL dichloromethane, shake horizontally for 10 min, centrifuge at 1439 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100  $\mu$ L n-hexane:isopropanol 75:25, centrifuge at 8160 g for 5 min, inject a 70  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Guard column:** 10  $\times$  4.5  $\mu$ m LiChrosorb RP8

**Column:** 250  $\times$  4.6 10  $\mu$ m Chiralpak AD

**Mobile phase:** n-Hexane:isopropanol 83.4:16.6

**Column temperature:** 28

**Flow rate:** 0.9 for 3 min, 0.65 for 7 min, 0.9 for 9 min, 1 for 2 min, 0.9 for 3 min

**Injection volume:** 70

**Detector:** UV 270 for 11.5 min, UV 305 for 1.65 min, UV 248 for 10.85 min

---

**CHROMATOGRAM**

**Retention time:** 12.55 (R), 16.44 (S)

**Internal standard:** R,S-propranolol (6.84), R-metoprolol (7.75), S-metoprolol (8.55)

**Limit of detection:** 5 ng/mL

**Limit of quantitation:** 10 ng/mL

---

**OTHER SUBSTANCES**

**Extracted:** metabolites

---

**KEY WORDS**

plasma; chiral

---

**REFERENCE**

Böhm, R.; Ellrich, R.; Koytchev, R. Quantitation of R- and S-propafenone and of the main metabolite in plasma, *Pharmazie*, **1995**, *50*, 542–545.

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Make serum alkaline with 10% sodium carbonate, extract with diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!). Remove the organic layer and extract it with 10 mM HCl, inject an aliquot of the aqueous layer.

---

**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil LC-CN

**Mobile phase:** MeCN:water:500 mM  $\text{KH}_2\text{PO}_4$  36:62:2

**Flow rate:** 1.8

**Detector:** UV 210

---

**CHROMATOGRAM**

**Limit of detection:** 5 ng/mL

---

**OTHER SUBSTANCES**

**Extracted:** metabolites, diltiazem, mexiletine

---

**KEY WORDS**

serum

---

**REFERENCE**

Kunicki,P.K.; Sitkiewicz,D. High-performance liquid chromatographic determination of some antiarrhythmic drugs using cyanopropyl derivatized silica phase (Abstract 43), *Ther.Drug Monit.*, **1995**, 17, 394–394.

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

---

**HPLC VARIABLES**

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 248

---

**CHROMATOGRAM**

**Retention time:** 7.38

**Limit of detection:** <120 ng/mL

---

**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celirolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; naproxen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetrizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demixiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opiipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide;

imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; caripramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 500  $\mu$ L Plasma + 100  $\mu$ L saturated sodium carbonate + 2 mL hexane: propanol:heptafluorobutanol 95:5:1.25, shake for 30 min, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L 0.005% R-(-)-1-(1-naphthyl)ethylisocyanate in hexane, vortex, let stand at room temperature for a short time, add 100  $\mu$ L bupranolol solution, evaporate to dryness, reconstitute in 200  $\mu$ L hexane, add 200  $\mu$ L 100 mM HCl, mix, centrifuge, inject a 100  $\mu$ L aliquot of the upper organic phase. (The bupranolol solution was obtained by dissolving 5 mg bupranolol hydrochloride in 1 mL water, adding 500  $\mu$ L saturated sodium carbonate, and extracting with 25 mL hexane. Use the hexane layer.)

## HPLC VARIABLES

**Column:** 150  $\times$  3.9 4  $\mu$ m 60 Å Nova-Pak silica

**Mobile phase:** Hexane:isopropanol:isobutanol 96:2:2

**Flow rate:** 1.5

**Injection volume:** 100

**Detector:** UV 220

## CHROMATOGRAM

**Retention time:** 7.5 (R-(-)), 9.5 (S(+))

**Internal standard:** bupranolol (16)

**Limit of detection:** 20 ng/mL

## KEY WORDS

plasma; chiral; derivatization; normal phase; pharmacokinetics

## REFERENCE

Volz,M.; Mitrovic,V.; Thieme,J.; Schlepper,M. Steady-state plasma kinetics of slow-release propafenone, its two isomers and its main metabolites, *Arzneimittelforschung*, **1995**, *45*, 246–249.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 211.1

---

#### CHROMATOGRAM

**Retention time:** 15.128

---

#### KEY WORDS

whole blood

---

#### REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

---

#### SAMPLE

**Matrix:** bulk

**Sample preparation:** Dissolve 10  $\mu$ mole compound (as free base or hydrochloride) in 500  $\mu$ L MeCN, add 250  $\mu$ L 5% sodium carbonate (for hydrochlorides only), add 500  $\mu$ L 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100  $\mu$ mole L-proline, heat at 60° for 30 min. Remove a 100  $\mu$ L aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10  $\mu$ L aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500  $\mu$ L 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°,  $[\alpha]_{546} = -133^\circ$  (c = 1) in MeCN).

---

#### HPLC VARIABLES

**Column:** 125  $\times$  4 5  $\mu$ m Hypersil ODS

**Mobile phase:** MeCN:20 mM ammonium acetate 70:30

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 254

---

#### CHROMATOGRAM

**Retention time:** k' 5.07, k' 5.98 (enantiomers)

---

#### KEY WORDS

derivatization; chiral

---

#### REFERENCE

Kleidermigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, 729, 33-42.

---

#### SAMPLE

**Matrix:** microsomal incubations

**Sample preparation:** 1 mL Microsomal incubation + 5 mL dichloromethane + 100  $\mu$ L 200  $\mu$ g/mL oxprenolol in MeOH, extract. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200  $\mu$ L mobile phase, inject an aliquot.

---

#### HPLC VARIABLES

**Column:** 150  $\times$  6 CLC-ODS (Shimadzu)

**Mobile phase:** MeCN:MeOH:15 mM sulfuric acid 32.5:4:63.5 containing 20 mM triethylamine

**Flow rate:** 1.5

**Detector:** UV 254

---

#### CHROMATOGRAM

**Retention time:** 9.8

**Internal standard:** oxprenolol (2.9)

---

#### OTHER SUBSTANCES

**Extracted:** metabolites, 5-hydroxypropafenone

---

#### KEY WORDS

mouse; liver

---

#### REFERENCE

Morita,K.; Mizuochi,M.; Yamaji,A.; Yokoyama,T. Stereoselectivity in the hydroxylation of propafenone enantiomers in mouse hepatic microsomes, *Biol.Pharm.Bull.*, **1994**, 17, 531–534.

---

#### SAMPLE

**Matrix:** solutions

**Sample preparation:** Mix a 50  $\mu\text{L}$  aliquot of a solution in MeOH:triethylamine 99:1 with 20  $\mu\text{L}$  0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50  $\mu\text{L}$  1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100  $\mu\text{L}$  mobile phase, sonicate for 30 s, inject a 20  $\mu\text{L}$  aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500  $\mu\text{L}$  triethylamine in 2 mL acetone dropwise, add a solution of 370  $\mu\text{L}$  ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10–15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93–94°), store in a desiccator under reduced pressure.)

---

#### HPLC VARIABLES

**Column:** 150  $\times$  3.9 4  $\mu\text{m}$  Nova Pak C18

**Mobile phase:** MeOH:water 70:30

**Flow rate:** 1

**Injection volume:** 20

**Detector:** F ex 296 em 356

---

#### CHROMATOGRAM

**Retention time:** 27.8 (R), 29.8 (S)

---

#### KEY WORDS

derivatization; chiral

---

#### REFERENCE

Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, 1, 223–234.

---

#### SAMPLE

**Matrix:** solutions

---

#### HPLC VARIABLES

**Guard column:** 30  $\times$  3.2 7  $\mu\text{m}$  SI 100 ODS (not commercially available)

**Column:** 150  $\times$  3.2 7  $\mu\text{m}$  SI 100 ODS (not commercially available)

**Mobile phase:** MeCN:buffer 31.2:68.8 (Buffer was 6.66 g  $\text{KH}_2\text{PO}_4$  and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

**Flow rate:** 0.5–1

**Detector:** UV 204, 244

---

**CHROMATOGRAM**

**Retention time:** 3.8

**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

---

**OTHER SUBSTANCES**

**Also analyzed:** aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

---

**REFERENCE**

Below, E.; Burrmann, M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J. Liq. Chromatogr.*, **1994**, *17*, 4131-4144.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

---

**CHROMATOGRAM**

**Retention time:** 12.20 (A), 6.37 (B)

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

**KEY WORDS**

details of plasma extraction

**REFERENCE**

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

**SAMPLE****Matrix:** solutions

**Sample preparation:** Mix 300  $\mu\text{L}$  of a 30  $\mu\text{M}$  solution in dichloromethane with 10  $\mu\text{L}$  20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50  $\mu\text{L}$  0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmole acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmole sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

**HPLC VARIABLES****Column:** 250  $\times$  4.5  $\mu\text{m}$  Zorbax ODS**Mobile phase:** MeCN:water 70:30**Flow rate:** 1**Injection volume:** 100**Detector:** UV 230, F ex 270 em 350**CHROMATOGRAM****Retention time:** k' 9.5 (R-(-)), 10.2 (S-(+))**OTHER SUBSTANCES****Simultaneous:** carvedilol, flecainide (no enantiomeric separation)**KEY WORDS**derivatization; chiral; F not much more sensitive than UV;  $\alpha = 1.07$ **REFERENCE**

Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, 725, 323–334.



# Propantheline bromide

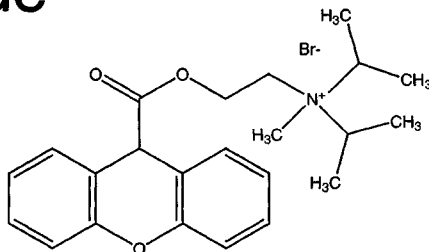
**Molecular formula:**  $C_{23}H_{30}BrNO_3$

**Molecular weight:** 448.40

**CAS Registry No.:** 50-34-0

**Merck Index:** 7989

**Lednicer No.:** 1 394



## SAMPLE

**Matrix:** bulk

**Sample preparation:** 50 mg Bulk drug + 5 mL 1.2% diethyl phthalate in MeCN + 45 mL MeCN, inject a 5  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 150  $\times$  3 TSK 410 (Toyo Soda)

**Mobile phase:** MeCN:50 mM pH 2.5 phosphate buffer 40:60

**Flow rate:** 1

**Injection volume:** 5

**Detector:** UV 258

## CHROMATOGRAM

**Retention time:** 5

**Internal standard:** diethyl phthalate (10)

## OTHER SUBSTANCES

**Simultaneous:** degradation products

## REFERENCE

Yoshioka, S.; Uchiyama, M. Kinetics and mechanism of the solid-state decomposition of propantheline bromide, *J.Pharm.Sci.*, **1986**, 75, 92-96.

## SAMPLE

**Matrix:** formulations

**Sample preparation:** Weigh out capsule contents, dissolve in 30 mL MeOH, add 1 mL 100  $\mu$ g/mL isothipendyl hydrochloride in MeOH, filter (paper), wash filter with MeOH, make up filtrate to 50 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 250  $\times$  4 5  $\mu$ m Econosphere C18

**Mobile phase:** MeOH:water:triethylamine 80:20:0.15, pH adjusted to 7.8 with phosphoric acid

**Flow rate:** 1.8

**Injection volume:** 10

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 9

**Internal standard:** isothipendyl (7)

**Limit of quantitation:** 60  $\mu$ g/mL

## OTHER SUBSTANCES

**Simultaneous:** haloperidol

## KEY WORDS

capsules

---

**REFERENCE**

Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, 29, 240-244.

---

**SAMPLE**

**Matrix:** membrane suspensions

**Sample preparation:** 200  $\mu$ L Membrane suspension + 10 mL ice-cold 1 mM pH 7.5 Tris-HCl buffer containing 150 mM NaCl, filter (0.45  $\mu$ m), wash filter with 15 mL ice-cold 1 mM pH 7.5 Tris-HCl buffer containing 150 mM NaCl. Remove the filter and add it to 4 mL 67 mM pH 7 phosphate buffer, heat in a boiling water bath for 40 min, remove the filter, add 500  $\mu$ L 1 M HCl to the solution, add 5 mL chloroform, shake gently for 15 min, centrifuge at 1500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200  $\mu$ L n-butyl p-aminobenzoate in MeOH, inject a 15-20  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.5  $\mu$ m Hitachi gel 3053 ODS

**Mobile phase:** MeCN:50 mM pH 2.5  $\text{KH}_2\text{PO}_4$  36:64

**Column temperature:** 55

**Flow rate:** 0.8

**Injection volume:** 15-20

**Detector:** UV 200

---

**CHROMATOGRAM**

**Retention time:** 10 (as xanthene 9-carboxylic acid)

**Internal standard:** n-butyl p-aminobenzoate (12)

---

**KEY WORDS**

propantheline is hydrolyzed to xanthene 9-carboxylic acid which is chromatographed

---

**REFERENCE**

Saitoh,H.; Kobayashi,Y.; Miyazaki,K.; Arita,T. A highly sensitive HPLC method for the assay of propantheline used to measure its uptake by rat intestinal brush border membrane vesicles, *J.Pharm.Pharmacol.*, **1987**, 39, 9-12.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-DP

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

---

**CHROMATOGRAM**

**Retention time:** 7.08

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordinazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-

azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazedol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimetoprim, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

---

**KEY WORDS**

details of plasma extraction

---

**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 50–200  $\mu$ L aliquot of a solution in pH 7.4 Tyrode's buffer.

---

**HPLC VARIABLES**

**Column:** 150  $\times$  3.9  $\mu$ m Nova-Pak C-18

**Mobile phase:** MeCN:50 mM phosphoric acid:triethylamine 40:60:0.1

**Column temperature:** 35

**Flow rate:** 0.6

**Injection volume:** 50–200

**Detector:** UV 230

---

**OTHER SUBSTANCES**

**Also analyzed:** chlorpromazine, verapamil

---

**KEY WORDS**

buffer

---

**REFERENCE**

Saitoh, H.; Aungst, B.J. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine, *Pharm. Res.*, **1995**, 12, 1304–1310.

# Proparacaine

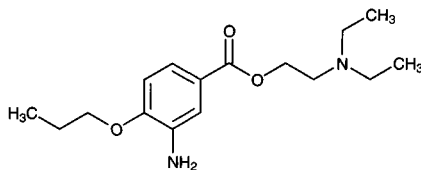
**Molecular formula:**  $C_{16}H_{26}N_2O_3$

**Molecular weight:** 294.39

**CAS Registry No.:** 499-67-2, 5875-06-9 (HCl)

**Merck Index:** 7991

**Lednicer No.:** 1 11



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu\text{g/mL}$  solution in MeOH, inject a 20  $\mu\text{L}$  aliquot.

## HPLC VARIABLES

**Column:** 125  $\times$  4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 2.9

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclimine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, mecllophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, naltrexone, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscipine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxylbenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thietilperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

---

**REFERENCE**

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191–225.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 150 × 4.5 5 µm Ultrasphere octyl

**Mobile phase:** MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35

**Column temperature:** 30

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 280

---

**CHROMATOGRAM**

**Retention time:** 2.33

**Internal standard:** naproxen (3.89)

---

**OTHER SUBSTANCES**

**Simultaneous:** bacitracin, cortisone acetate, diazepam, diclofenac, fluorometholone, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, neomycin, prednisolone acetate, salicylic acid, sulfacetamide, suprofen

**Noninterfering:** acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide

**Interfering:** metipranolol, propranolol

---

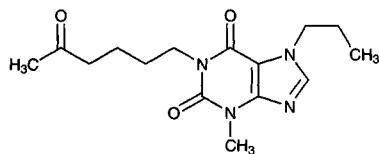
**REFERENCE**

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, 654, 140–145.

---

---

# Propentofylline



**Molecular formula:** C<sub>15</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>

**Molecular weight:** 306.36

**CAS Registry No.:** 55242-55-2

**Merck Index:** 7997

---

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 100 µL Plasma + 100 µL 20 µg/mL tetracaine hydrochloride + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 µL mobile phase, inject a 20 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 150 × 6 Shimpack CLS-ODS (Shimadzu)

**Mobile phase:** MeCN:MeOH:0.5 mM phosphoric acid 21:20:59

**Column temperature:** 40

**Flow rate:** 1.5

**Injection volume:** 20

**Detector:** UV 273

---

**CHROMATOGRAM****Internal standard:** tetracaine hydrochloride**KEY WORDS**

plasma; rat

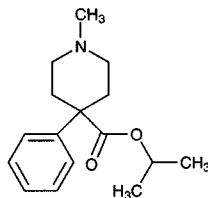
**REFERENCE**

Lee, C.K.; Uchida, T.; Kitagawa, K.; Yagi, A.; Kim, N.-S.; Goto, S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, 83, 562–565.

**SAMPLE****Matrix:** solutions**Sample preparation:** Inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 150 × 6.5 µm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)**Mobile phase:** MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)**Column temperature:** 30**Flow rate:** 1**Injection volume:** 20**Detector:** UV 270**CHROMATOGRAM****Retention time:** 18**Internal standard:** 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)**OTHER SUBSTANCES****Simultaneous:** caffeine, hypoxanthine, pentoxifylline, theobromine, theophylline, uric acid, xanthine**REFERENCE**

Nakashima, K.; Inoue, K.; Mayahara, K.; Kuroda, N.; Hamachi, Y.; Akiyama, S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, 722, 107–113.

# Properidine

**Molecular formula:** C<sub>16</sub>H<sub>23</sub>NO<sub>2</sub>**Molecular weight:** 261.36**CAS Registry No.:** 561-76-2**Lednicer No.:** 1 299**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

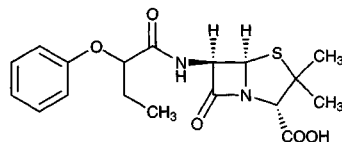
**CHROMATOGRAM****Retention time:** 3.1**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, diphenhydramine, diphenoxylate, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cycizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipranone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscipine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

# Propicillin

**Molecular formula:** C<sub>18</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub>S**Molecular weight:** 378.45**CAS Registry No.:** 551-27-9, 1245-44-9 (potassium salt)**Merck Index:** 8002**SAMPLE****Matrix:** perfusate**Sample preparation:** Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.**HPLC VARIABLES****Guard column:** 20 × 2.5 μm Hypersil ODS

**Column:** 150 × 4.6 5 µm Hypersil ODS  
**Mobile phase:** MeCN:50 mM pH 7 KH<sub>2</sub>PO<sub>4</sub> buffer 30:70  
**Flow rate:** 1  
**Injection volume:** 100  
**Detector:** UV 218

---

**CHROMATOGRAM**

**Retention time:** 4.1  
**Limit of detection:** 20 ng/mL  
**Limit of quantitation:** 100 ng/mL

---

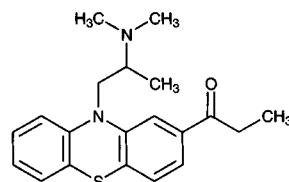
**REFERENCE**

Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, 705, 63–69.

---

# Propiomazine

**Molecular formula:** C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>OS  
**Molecular weight:** 340.49  
**CAS Registry No.:** 362-29-8, 1240-15-9 (HCl)  
**Merck Index:** 8007  
**Lednicer No.:** 1 376



---

**SAMPLE**

**Matrix:** solutions  
**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica  
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7  
**Flow rate:** 2  
**Injection volume:** 20  
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

---

**CHROMATOGRAM**

**Retention time:** 2.7

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethiopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine,